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Neuroendocrinelike (Small Granule) Epithelial Cells of the Lung

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The presence of neuroendocrinelike epithelial cells in the lung of numerous species has been demonstrated by light and electron microscopy. Histochemical methods used to identify these cells have included staining with silver, amine-type fluorescence (APUD cell), periodic acid Schiff (PAS)–lead hematoxylin, and immunohistochemical localization of neuron-specific enolase. Cytoplasmic dense core vesicles (70–200 nm in diameter) have served as the major ultrastructural characteristic. Lung neuroendocrinelike cells have been shown to occur in fetal and adult mammals as solitary-type cells or as distinct organoids known as neuroepithelial bodies (NEBs). Although the frequency of both populations is considered low, solitary-type cells with dense-core granules can be found in as high as 5% of epithelial cells in the cricoid region of the guinea-pig larynx. The solitary cells can be found throughout the airways of mammals, whereas the NEBs are confined to the intrapulmonary airways. Unmyelinated fibers have been traced from the lamina propria and into the NEB, where they ramified between the component cells of the NEB. The function of lung neuroendocrinelike cells is not known, but morphological and cytochemical studies suggest that the NEBs are intrapulmonary chemoreceptors that can respond to changes in airway gas composition. Hypoxia or hypercapnia has been shown to decrease the amine cytofluorescence in these organoids and apparently to increase the exocytosis of dense core vesicles from the basal region of the cell. Immunohistochemical studies have suggested that some lung epithelial cells may contain a known neuropeptide(s), but further investigation is needed to confirm the presence of such compounds in lung neuroendocrinelike cells and their physicochemical properties. Apparent hyperplasia of lung neuroendocrinelike cells can occur readily in hamsters treated with diethylnitrosamine. It has been postulated that human lung tumors with endocrinelike properties, namely, bronchial carcinoids and lung small cell carcinomas, may originate from lung neuroendocrinelike cells. However, a more plausible explanation, based on cytokinetic studies of epithelial neuroendocrinelike cells in the lung and other organs, is that these cells originate from a nonneuroendocrine population. Interaction of such a progenitor cell population with selected carcinogens may lead to stimulation of the rate of normal differentiation or, alternately, to selection of an abnormal route of differentiation that possesses a neuroendocrine phenotype.

Knowledge of the structural and functional aspects of individual cell types of the lung is important in assessing toxic reactions which may be caused by bloodborne chemicals or inhaled gases or particulates. In this report, we have attempted to provide a comprehensive and critical review of lung epithelial neuroendocrine cells. Although their extremely low numbers discourage investigation, these cells probably have vital regulatory roles in lung function and participate in responses to different classes of toxic agents.

Morphological studies of lung epithelial neuroendocrinelike cells have shown that they occur as single cells sparsely distributed throughout the tracheobronchial epithelium or as component cells of the lung neuroepithelial body (NEB), a distinct organoid which appears innervated and perhaps restricted to the intrapulmonary airways. Both solitary neuroendocrinelike cells and NEBs are found (*1–88*) in the lungs of humans and other species (Table 1).

Nomenclature

The various names given to these cells are derived from morphological or cytochemical characteristics. Frölich (*38*) referred to the solitary cells as "Helle-Zellen," or "clear cells," due to their poor staining by various dyes. These cells have also been referred to as Feyrter cells (*44*), after the Austrian pathologist who described them as early as 1938 (*89*); other names include Kultschitzky-like (*13*), or K cells, AFG (argyrophil, fluorescent and granulated) cells (*90*), small-granule endocrine cells (*22*), endocrinelike cells (*1,49*), APUD cells (*4*), P cells (*91*), enterochromaffinlike cells (*58*), neurosecretory-appearing cells (*16*), or more recently, neuroendocrine cells (*33*). Except when it is necessary to deal with the solitary neuroendocrine cells or the NEB independently, we shall refer to them collectively as neuroendocrine cells; solitary neuroendocrine cells will be referred to as K cells (*92*). The current status of

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Table 1. Cytochemical, ultrastructural and functional studies of lung neuroendocrine cells in man and animals.

Species	Methods[a]	References
Man		
Fetus	Ag, APUD, FIF, EM, NSE, AChE, innervation	(*1–8*)
Newborn	Ag, FIF, EM	(*2,5,8–11*)
Child	Ag, FIF, EM	(*2,12*)
Adult	Ag, APUD, FIF, EM, NSE	(*4,5,7,12–16*)
Monkey	Ag, ozone exposure	(*17,18*)
Sheep		
Fetal	Ag, FIF, EM, CA, AChE	(*2,19,20*)
Adult	Ag, FIF, CA, AChE	(*2,19,20*)
Calf	CA, AChE	(*19,20*)
Goat	CA, AChE	(*19,20*)
Pig	Ag, CA, AChE	(*18,20*)
Lion	Ag	(*18*)
Cat	Ag, EM, innervation, PAS–Pb–He	(*12,21–23*)
Rabbit		
Fetal	Ag, FIF, EM, SEM, APUD, ^{3}H-thymidine incorporation, 5-HT, CA, AChE, PAS–Pb–He, hypoxia, innervation, quantitation, organ culture	(*2,18,20,21,24–32*)
Newborn	Ag, APUD, FIF, EM, CA, AChE, ^{3}H-thymidine incorporation, hypoxia, hyperoxia, hypercapnia, nicotine, reserpine	(*18,20,25,27,29,33–36*)
Adult	Ag, APUD, FIF, EM, 5-HT, hypoxia, hyperoxia, hypercapnea	(*18,20,29,34,37,38*)
Guinea pig		
Fetal	APUD, FIF	(*25*)
Adult	Ag	(*39*)
Hamster	Ag, APUD, FIF, EM, PAS–Pb–He, NO_2 exposure, quantitation, DEN exposure	(*22,40–47*)
Rat		
Fetal	Ag, APUD, FIF, EM	(*48*)
Adult	Ag, APUD, FIF, EM, CA, AChE, asbestos exposure, quartz dust, coal dust exposure	(*22,40,49–54*)
Mouse		
Fetal	Ag, APUD, FIF	(*25*)
Newborn	Ag, FIF, EM, SEM, hypoxia, NO_2 exposure	(*22,34,55,56*)
Adult	Ag, APUD, FIF, EM, innervation	(*47,57–59*)
Badger	Ag	(*18*)
Hedgehog	Ag	(*18*)
Chicken		
Embryo/Adult	Ag, APUD, FIF, EM, CO_2 receptors, innervation	(*60–66*)
Duck	CO_2 receptors	(*67–69*)
Goose	CO_2 receptors	(*70*)
Armadillo	Ag, FIF, EM	(*2*)
Turtle	CO_2 receptors	(*71*)
Lizard	5-HT, CA, innervation	(*72*)
Snake	EM	(*71,73*)
Toad	FIF, EM, innervation	(*74,75*)
Frog	Ag, FIF, CO_2 receptors	(*76,77*)

[a] Methods: Ag = argentaffin and/or argyrophilic silver (*39,78–81*); APUD = amine precursor uptake and decarboxylation characteristics (*82*); FIF = formaldehyde-induced fluorescence (*20,30,81,83,84*); EM = ultrastructural examination (*81,85,86*); NSE = neuron-specific enolase (*7,87*); AChE = acetylcholinesterase (*84*); CA = catecholamines (*20,83,84*); PAS-Pb-He = PAS-lead-hematoxylin (*22,88*); SEM = scanning electron microscopy (*24,56,75*); 5-HT = serotonin (*29,30*); DEN = diethylnitrosamine (*50,52–54*).

information pertaining to the cytochemical and morphological properties and responsiveness of lung neuroendocrine cells to selected pharmacological agents, as discussed below, seems to give adequate justification for provisional use of the term "neuroendocrine" in this review.

Morphology

Both light and electron microscopic studies have shown that K cells are usually flask-, oval- or pyramidal-shaped with most of the cytoplasm concentrated at the base of the cells next to the basal lamina (*10,15,22,25,48,58,93,94*). Occasionally, lateral dendritelike cytoplasmic processes extend between nearby epithelial cells (*10,13,15,94*). The irregular path of the apical region of the cell has made it difficult to ascertain in a single section whether these cells reach the lumen; where this occurred, microvilli were revealed on the cell surface (*14–16,25,40,48,58*). The identification of small, cytoplasmic, generally spherical granules, otherwise known as dense-core vesicles (DCVs), has served as the major ultrastructural characteristic of the K cell (Figs. 1–3). The size and appearance of the granules vary among species, where diameters of these organelles can range from 70 to 200 nm. The granules contain a central core of variable electron density that is generally

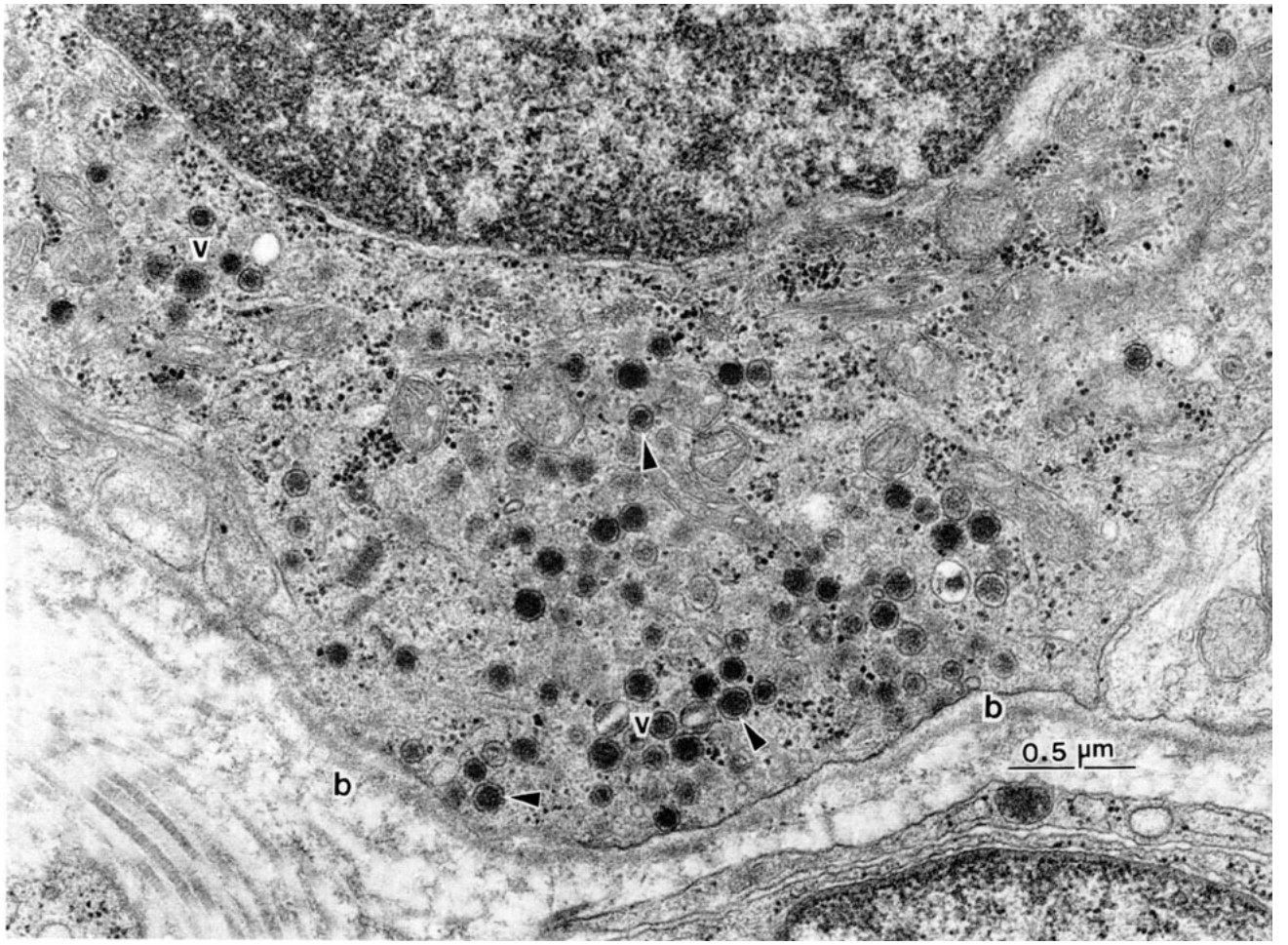

FIGURE 1. Small, spherical granules (v) of variable density in the basal cytoplasm (basal lamina = b) of a solitary, epithelial neuroendocrine cell (K cell) in the cricoid region of the adult mouse larynx. A narrow perigranular "halo" (arrows) can be seen next to a surrounding trilaminar membrane. TEM ×33,000.

separated from the surrounding trilaminar membrane by a thin, electron-lucent zone or "halo." The granules are observed throughout the cytoplasm but, according to some workers, appear to concentrate in the perinuclear and basal cytoplasm. In some species, differences are observed in the morphology of the granules among individual K cells. Hage (*3,95*), for instance, has described three types of K cells in human fetal lungs, each with a particular kind of granule and cytochemistry. The armadillo and lamb (Fig. 3) have been found to contain at least two populations of K cells based on the appearance and dimensions of the small granules (*2*). The adult rabbit (*2*), human (*95*) and guinea pig (*96*) revealed (Fig. 2) only one population.

Other ultrastructural characteristics associated with K cells are variable amounts of free ribosomes and mitochondria; the latter are usually smaller than those of adjacent cells (*48,58*). The Golgi apparatus is usually visible and present in a supranuclear position (Fig. 2). Bundles of cytoplasmic filaments, smooth and rough endoplasmic reticulum and rosettes of glycogen are usually found in variable amounts. Electron micrographs of some K cells exhibit pleomorphic, lysosomelike inclusions or dense bodies (Fig. 2). Whorls of membrane, possibly derived from small granules, are occasionally observed in these organelles. Although submucosal and intraepithelial nerve fibers are commonly observed in the lung (*97–99*), fine structural analyses of the K cell have failed to demonstrate any specific contact by nerves or synaptic processes (*2,13,16,25, 48,94*).

Except for the corpuscular arrangement of neuroendocrine cells that constitute the NEB (Fig. 4), the ultrastructural features of the individual cells of this organoid are similar to those of the K cell (Figs. 5–9). In fetal rabbit NEBs (Fig. 4), Lauweryns et al. (*18,100*) and Sonstegard et al. (*31*) reported that the DCVs of the component cells exist mainly as two types within the same cell (Fig. 5). The type 1 DCVs are wedge- or oval-shaped with a dense, amorphous core and a diame-

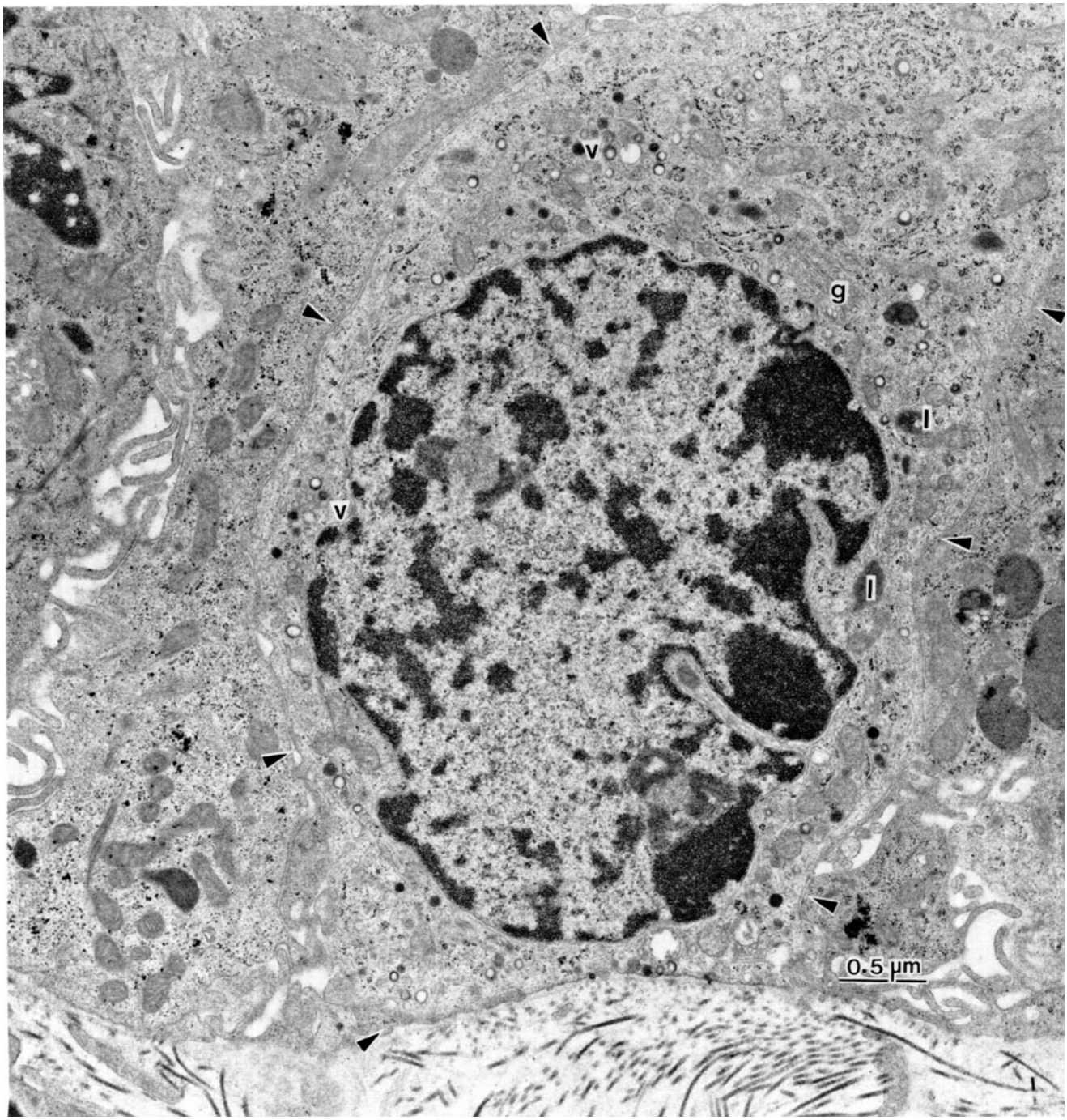

FIGURE 2. Guinea pig tracheal K cell (arrows). The neuroendocrinelike granules, or vesicles (v), have a diameter of 130 ± 25 nm and variable morphology. Some granules have a spherical, electron-lucent region in the electron-dense central core. Granules may appear "empty," revealing only the outer trilaminar membrane. *Lysosomelike dense body* (l) and Golgi apparatus (g). TEM. ×15,000.

ter approximately 136 nm. There is usually *no* halo between the dense core and the membrane. In contrast, the type 2 DCVs are approximately 105 nm in diameter, have a more circular shape, and exhibit a less electron-dense core which is surrounded by a distinct halo of about 15 to 20 nm. "Empty" NEB cell vesicles are occasionally observed in direct contact with the extracellular space (*34*). Recently, Sonstegard et al. (*101*) found that individual cells of fetal rabbit NEBs had the two types of granules as earlier reported but in the same lungs there were NEBs whose cells contained only larger (approximately 148 nm diameter) enterochro-

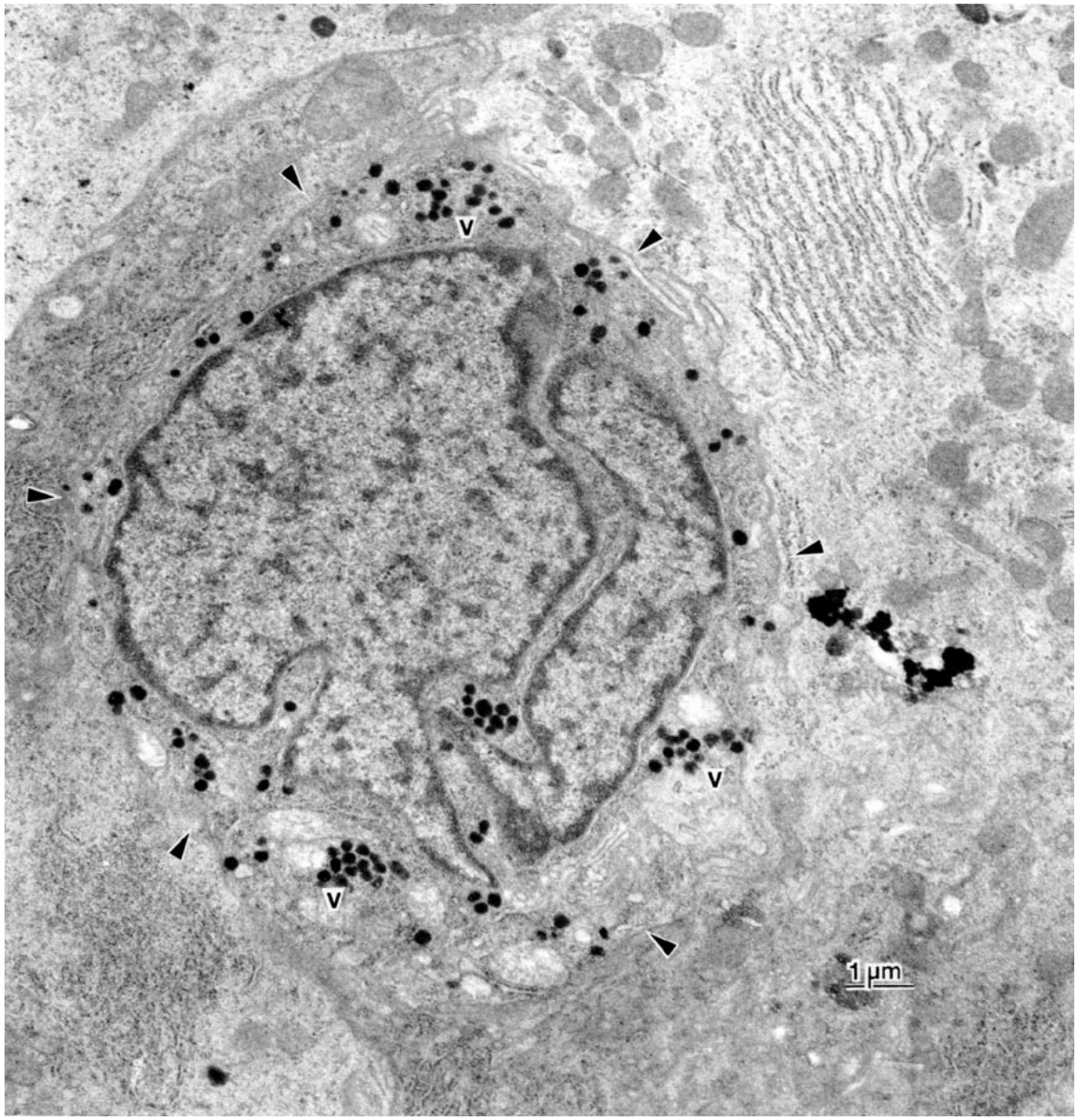

FIGURE 3. Tangential section through a tracheal K cell (arrows) in newborn lamb showing electron-dense, irregularly shaped secretory granules (v) of the neuroendocrine-type with a very narrow perigranular halo. TEM. ×20,790.

maffinlike granules (Fig. 6). Variation in the morphology of the DCVs within individual cells has been interpreted to represent various stages in their formation or secretion (*18,31*).

NEB-like structures composed of a few to as many as 80 cells have been found in the intrapulmonary airways of near-term fetal rabbit lungs (*24*) (Fig. 4). The NEB consists of parallel, nonciliated cylindrical cells that contact the basal lamina and may extend to the lumen (*100*) (Figs. 4 and 7). The basal aspect of the NEB may protrude into the underlying submucosa. The lateral boundaries of NEB cells are usually characterized by complex interdigitation, desmosomal connections and occasionally primary cilia; the apical boundaries are organized by junctional complexes consisting of tight junctions and desmosomes (*24*) (Figs. 4 and 7). The surface area of NEBs exposed to the airway lumen appears to be species specific (Figs. 4 and 7). The exposed surface of NEB cells exhibit short microvilli (*18,24*). In the toad (*B. marinus*), the majority of neuroendocrine cells does not make direct contact with the lumen, but instead, is covered by an apical cell (*75*). A striking feature of this cell is that it has a cilium located at the luminal surface with an 8 & 1 microtubu-

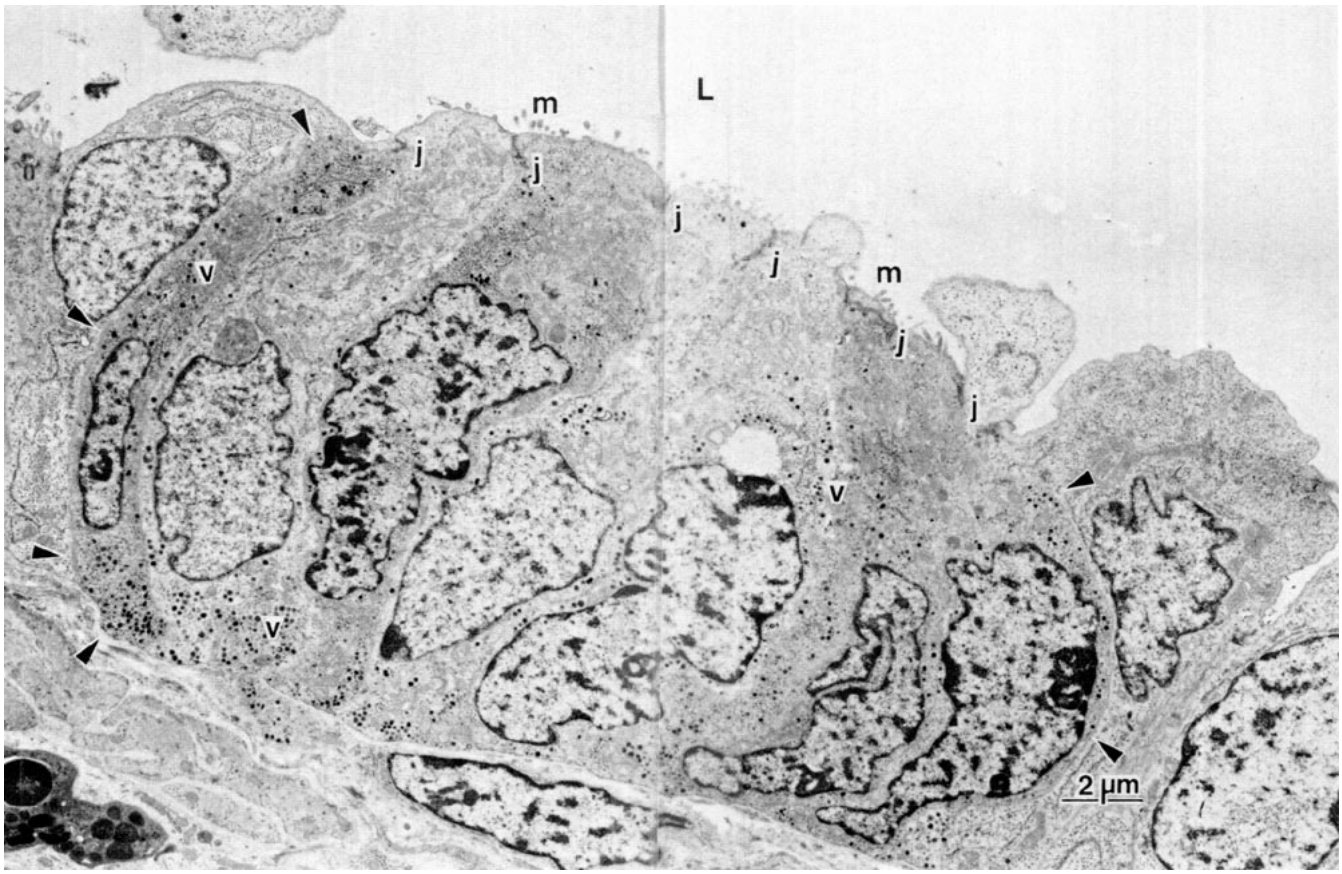

FIGURE 4. Neuroepithelial body (arrows) in 29 day fetal rabbit lung airway mucosa. Several colmnar cells each contain neuroendocrinelike, dense-core vesicles (v). The exposed surface of the NEB is covered by microvilli (m) and tight junctions (j) are prominent at the cell surface bordering the lumen (L) TEM. ×5580.

lar arrangement, suggesting that it is of the sensory nonmotile type. A scanning electron microscopic study by Cutz et al. (*24*) showed that the surfaces of rabbit fetal NEBs were partially covered by nonciliated cells with a dome-shaped apical cytoplasm similar to that of Clara cells. The unique topographical characteristics of the fetal rabbit NEB as viewed from the airways by scanning electron microscopy are shown in Figure 10. NEBs appear in fetal rabbit lung by day 19 of gestation; ciliated cells appear prior to term, i.e., day 27, but Clara cells with granules have not been observed in prenatal rabbit lung (*32,101*). Sonstegard et al. (*101*) found that the dome-shaped cells adjacent to the fetal rabbit NEBs (Fig. 4) did not contain Clara cell-like granules or large amounts of smooth endoplasmic reticulum, but, instead, contained large deposits of glycogen. It is possible that these cells differentiate to Clara cells. Sorokin et al. (*32*) recently examined the development of NEBs in fetal rabbit lungs and proposed that these organoids arise from precursors ("clear cells") that first appear in the endodermal epithelium of pseudoglandular lungs around 18 to 19 days of gestation. Hung et al. (*55,56,59*) examined the neonatal mouse lung by transmission and scanning electron microscopy and found that the boundary of NEBs was outlined by ciliated cells and Clara cells, with "modified" Clara cells covering most of the surface of the NEBs. The relationship of ciliated cells or Clara cells to NEBs remains unclear (*24,55,56*).

Innervation of the NEB has been demonstrated by light and electron microscopy and serves as a key morphological trait to identify these organoids in the lung epithelium (*34,55,59,74*). Lauweryns and Cokelaere (*34*) found that the cell cytoplasm and nerve endings in rabbit NEBs stained for acetylcholinesterase. Cutz and Orange (*102*) reported a dense network of acetylcholinesterase-positive fibers within an NEB in lamb bronchiolar mucosa. Unmyelinated fibers have been traced from the lamina propria and into the NEB in newborn humans (*11*), fetal rabbits (*100*), and neonatal mice (*55*) where the fibers ramified between granulated cells. It has been proposed that the NEB is innervated by both afferent and efferent fibers (*34,74*). Rogers and Haller (*74*) divided toad NEBs on the basis of innervation;

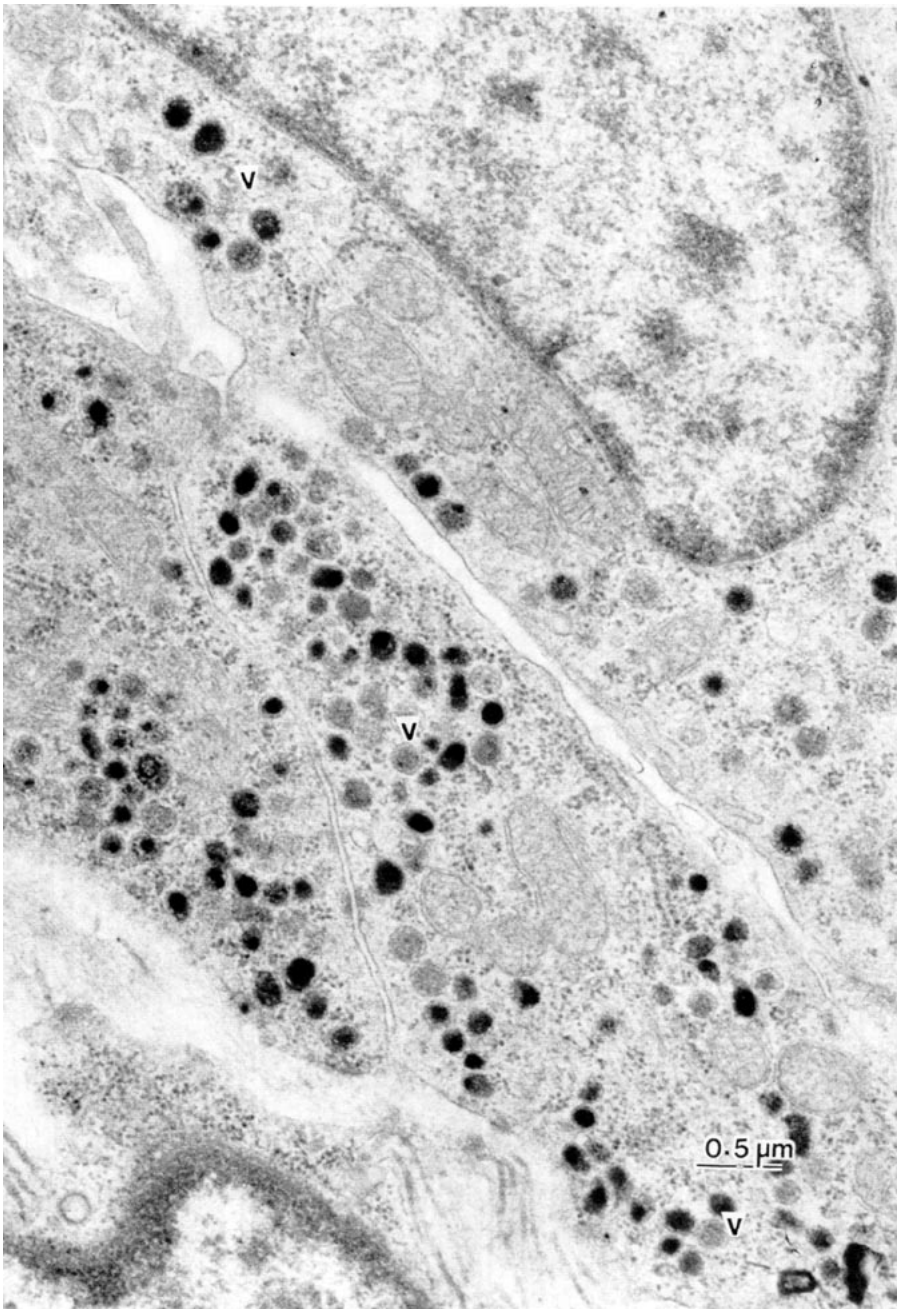

FIGURE 5. *Dense-core vesicles (v) in the basal cytoplasm of a neuroepithelial body in 29-day fetal rabbit lung. TEM.* ×26,880.

about 60% were innervated solely by nerve fibers containing agranular vesicles which form reciprocal synapses; about 20% were innervated solely by adrenergic nerve fibers which form distinct synaptic contacts; and the remaining 20% were innervated by both types of nerve fibers.

The nerve fibers within rabbit NEBs are characterized by their electronlucent axoplasm, neurotubules, and small mitochondria (*34*). Some of these fibers have been reported to form synapses with the NEB granulated cells. At the site of contact, synaptosomes were present that contained agranular vesicles between 30 and 50 nm in diameter. In some instances the apparent exocytosis of DCVs has been observed at the site of contact between an afferentlike nerve ending and a granulated cell. Lauweryns and Cokelaere (*34*) have given a detailed description of the ultrastructure of the different types of nerve endings in contact with NEB cells. The cellular organization, ultrastructure, and innervation of the NEB suggest an anatomical homolog

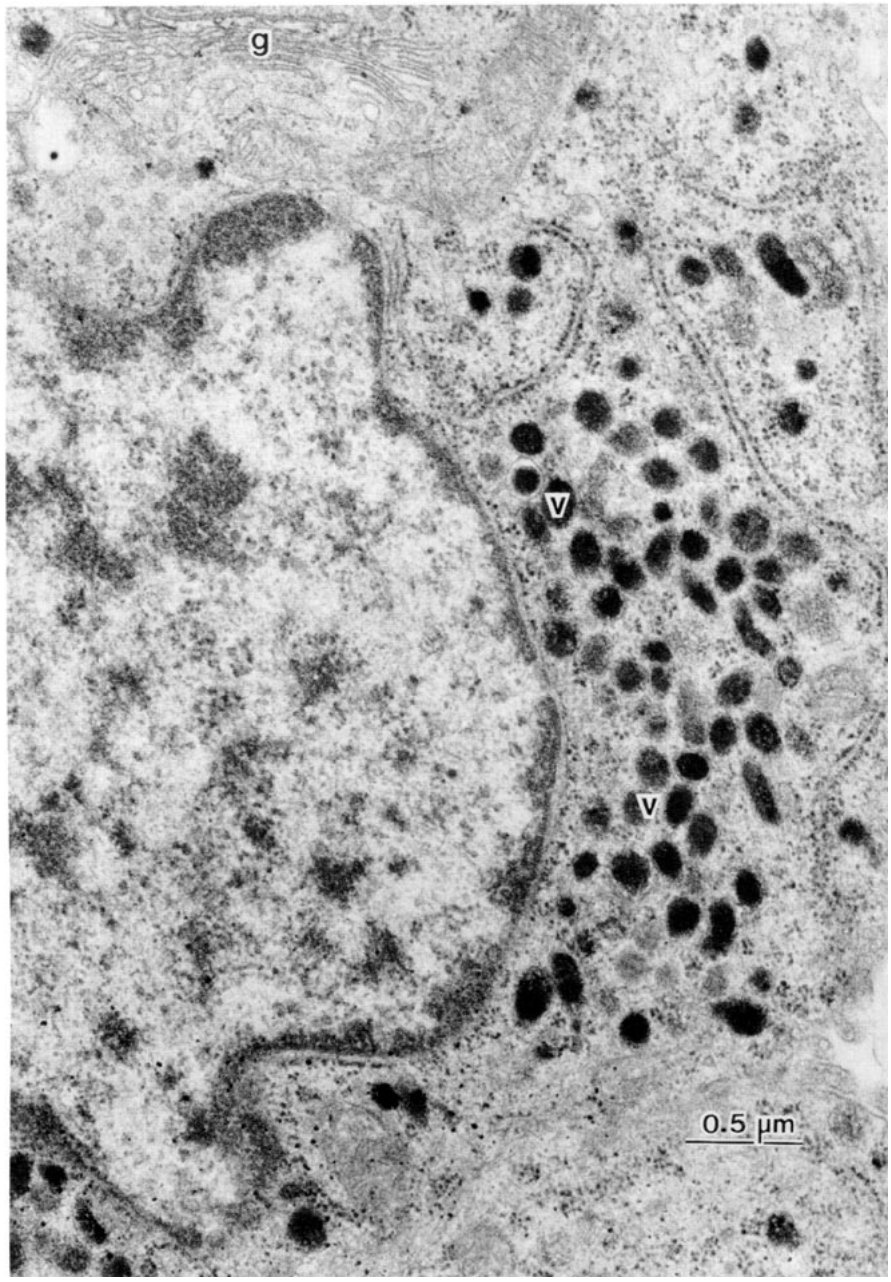

FIGURE 6. Enterochromaffinlike, dense-core vesicles (v) and Golgi apparatus (g) in cells comprising some neuroepithelial bodies in late gestation rabbit airway mucosa. TEM. ×33,000.

of the taste bud and the carotid body, known peripheral chemoreceptors. This makes it reasonable to propose that common functional properties, as well, may be found among these neuroendocrine organoids. This aspect will be discussed in the section on "Function."

Cytochemistry

The cytochemical procedures used to identify lung neuroendocrine cells are given in Table 1. The localization of specific polypeptide hormones by immunocytochemical methods will be discussed in the "Peptides" section of this review. Prior to their use on lung tissue, most of the cytochemical and specific-antibody stains have found use in the identification of neuroendocrine cells in other organs such as the gastrointestinal tract, pituitary and adrenal medulla. Therefore, it is not unreasonable to expect that the cytochemical and immunocytochemical reactions used to demonstrate the K cells can be used for the NEB and *vice versa*. The

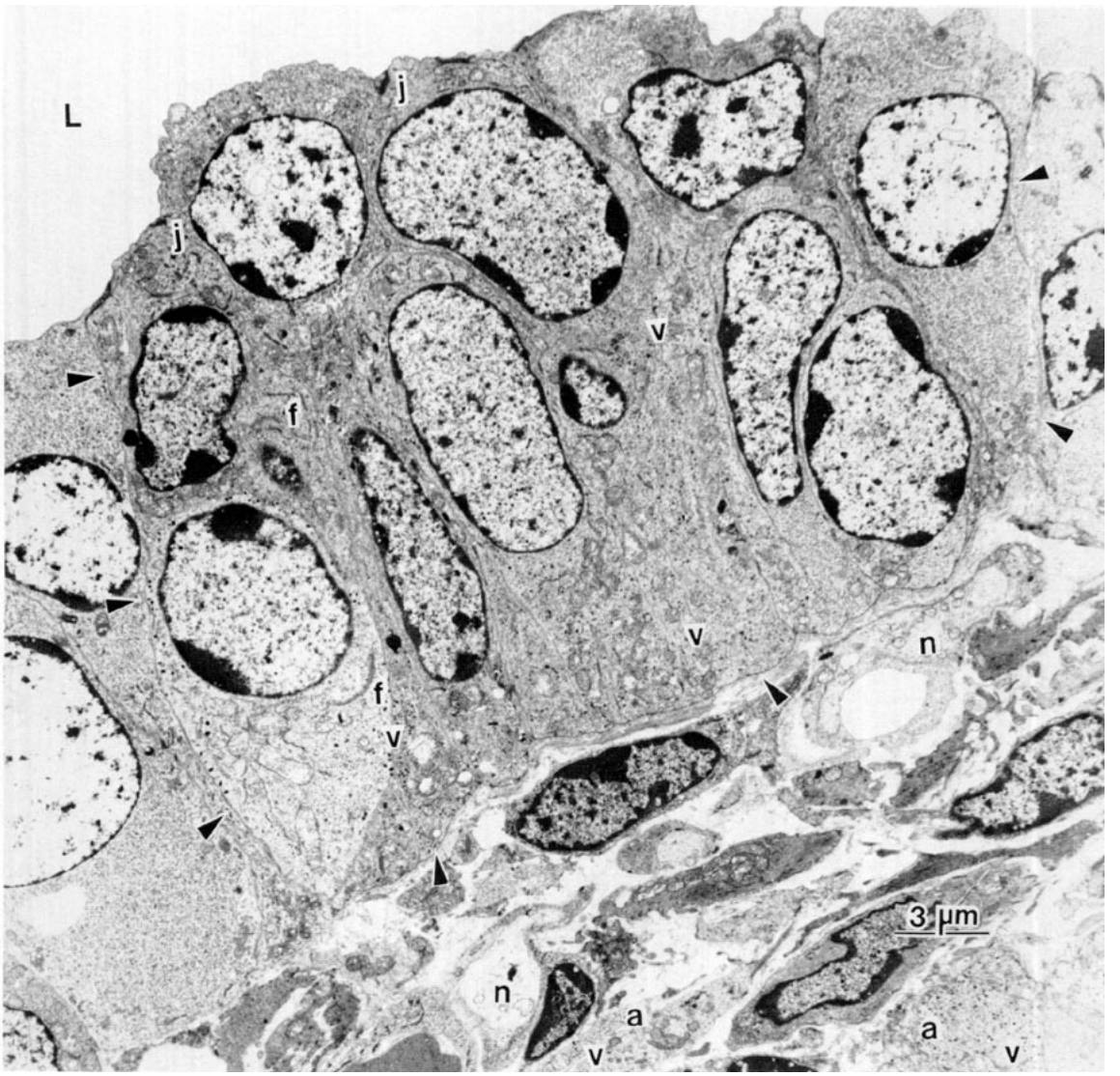

FIGURE 7. A group of near-term fetal hamster airway epithelial cells (arrows) contain neuroendocrinelike, dense-core vesicles (v), bundles of filaments (f) and tight junctions (j). Nerve bundles (n) with myelinated and unmyelinated fibers and large axon terminals (a) with numerous dense-core vesicles reside in the submucosa; lumen (L). TEM. ×4690.

corpuscular arrangement of NEB cells makes these structures apparent in H & E-stained sections or 1 µm thick plastic sections stained with toluidine blue (*100*) (Fig. 11). However, conventional stains do not seem to permit the identification of individual lung neuroendocrine cells; therefore, the development of specific and convenient stains remains a worthwhile pursuit. Even when specific staining is indicated, it is important to consider, especially in the case of the solitary neuroendocrine cells, whether the stain in a given region of the airways reveals all the epithelial neuroendocrine cells or just a select population. This is why investigators should use various cytochemical methods, especially in those types of studies where the environment of these cells may be altered. For example, a cytochemical reaction dependent on a biogenic amine may be inappropriate if the amine is secreted or otherwise depleted during the experiment.

Silver impregnation was one of the first methods used to identify lung neuroendocrine cells and caused Feyrter (*103*) to propose a "peripheral endocrinic (paracrinic)" system for the lung and other organs. The specific substances or types of compounds reacting with silver have not been identified, although silver staining appears to be associated with the secretory granules of the neuroendocrine cells (*58*). Both K cells and NEBs (Fig. 12) have been stained by this method; the presence of positive cells, however, may be dependent on the species and age of the animal. Argyrophilic, but not argentaffin-staining, epithelial cells have been demon-

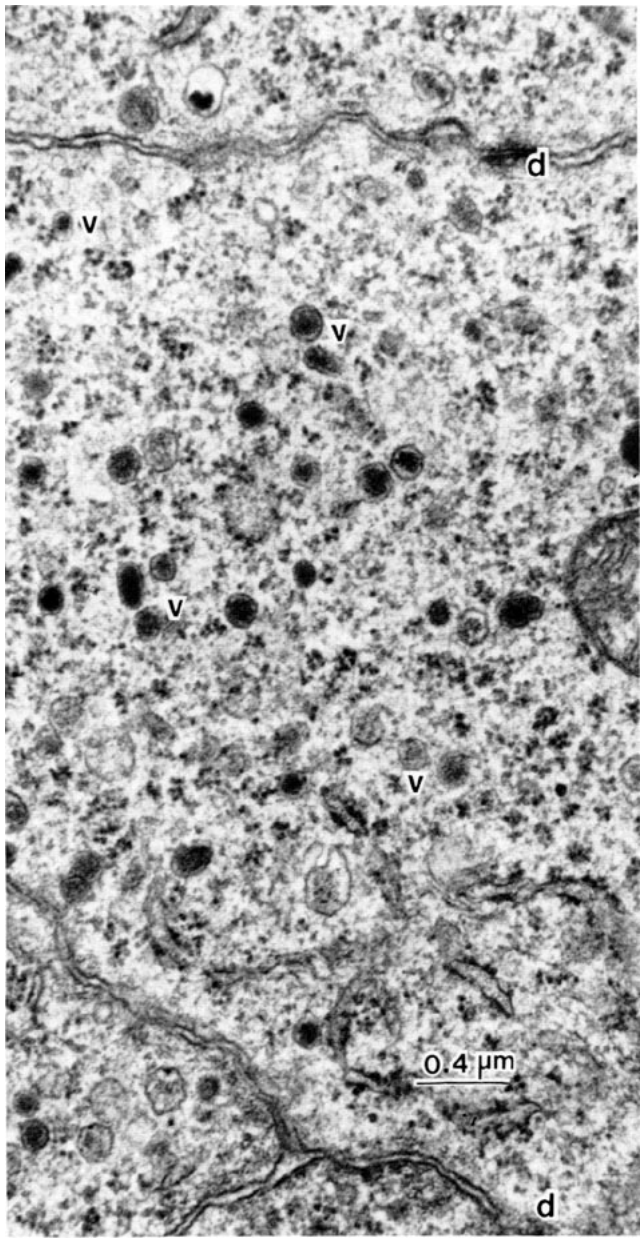

FIGURE 8. Neuroendocrinelike, dense-core vesicles (v) of variable shape and electron-density in the cytoplasm of cells comrpising the 17-day fetal hamster lung neuroepithelial body. The lateral boundaries of the cells are connected by desosomes (d). TEM. ×30,660.

strated in human fetuses and adults (*104*).* Fetal or neonatal rabbit NEBs are both argyrophilic and argentaffin-staining (*18,101*).

The formaldehyde-induced fluorescence (FIF) of biogenic monoamines (*105,106*) is another method used to identify lung neuroendocrine cells. This reaction is based on the capacity of these cells to store catecholamines or indolealkylamines or convert the amine precursor, 5-hydroxytryptophan (5-HTP) or L-dihydroxyphenylalanine (L-DOPA) to serotonin or dopamine, respectively. By possessing these cytochemical characteristics, lung neuroendocrine cells are classified as members of the APUD (amine precursor uptake and decarboxylation) cell system, which represents a diverse group of neuroendocrine cells with common ultrastructural and cytochemical properties (*91*). Many of the neuroendocrine cells in the APUD series are known to contain polypeptide hormones. Recent studies indicate that biogenic amines and polypeptide hormones are stored in the same dense-core vesicles and are released simultaneously (*107–109*). It is inferred that these same properties extend to lung neuroendocrine cells that exhibit amine-type fluorescence. There are lung epithelial cells in some species that contain enough endogenous biogenic amine to permit detection without prior administration of the amine precursor. NEBs of fetal or neonatal rabbits, for instance, reveal yellow fluorescent cells indicative of serotonin (*29*) (Fig. 13). Serotonin in fetal rabbit NEBs can also be demonstrated immunocytochemically with antiserum to this amine (*101*) (Fig. 14). Enriched cell fractions containing isolated fetal rabbit NEBs revealed the presence of serotonin and dopamine by high performance liquid chromatography (*101*). Yellow fluorescent cells have also been demonstrated in the primary bronchus of the frog (*66,77*). Other lung neuroendocrine cells may normally contain the amine at a concentration below the level of detection but exhibit FIF after administration of the amine precursor (*2,4,51*). It is conceivable that some neuroendocrine cell populations may have an unidentified amine that is not visualized by current FIF techniques or that they may lack altogether the capacity to synthesize or store biogenic amines. Eaton and Fedde (*57*) identified two distinct populations of amine-containing cells in the mouse lung without preincubation with amine precursors; one population emitted a yellow fluorescence and the other a yellow-green fluorescence, which was possibly due to dopamine or norepinephrine. However, yellow fluorescence is the most often reported emission in lung neuroendocrine cells (*18,29*). Hage (*4*) reported that some cells of the bronchial mucosa of human fetuses exhibited a green fluorescence. Dey et al. (*93*) reported that rabbit tracheal epithelium contained solitary yellow fluorescent cells that were argentaffin, argyrophilic, and ferric ferricyanide-positive. The number of fluorescent cells was reduced after reserpine administration but was not affected by injecting L-DOPA. Ericson et al. (*58*) reported that neuroendocrine cells of the mouse trachea accumulate radioactivity after administration of [^{3}H-]DOPA or [^{3}H-]5-HTP as revealed by autoradiography. The labeling was associated with the cytoplasmic, electron-dense granules, suggesting to the authors that amine formation occurred in these granules.

A recently developed cytochemical method used fluorescamine, a compound that forms intensely fluores-

*Argyrophilic cells require an exogenous reducing agent to stain with silver; argentaffin cells stain with silver without the addition of a reducing agent. Argentaffin cells are usually argyrophilic, whereas argyrophilic cells are not always argentaffin-staining.

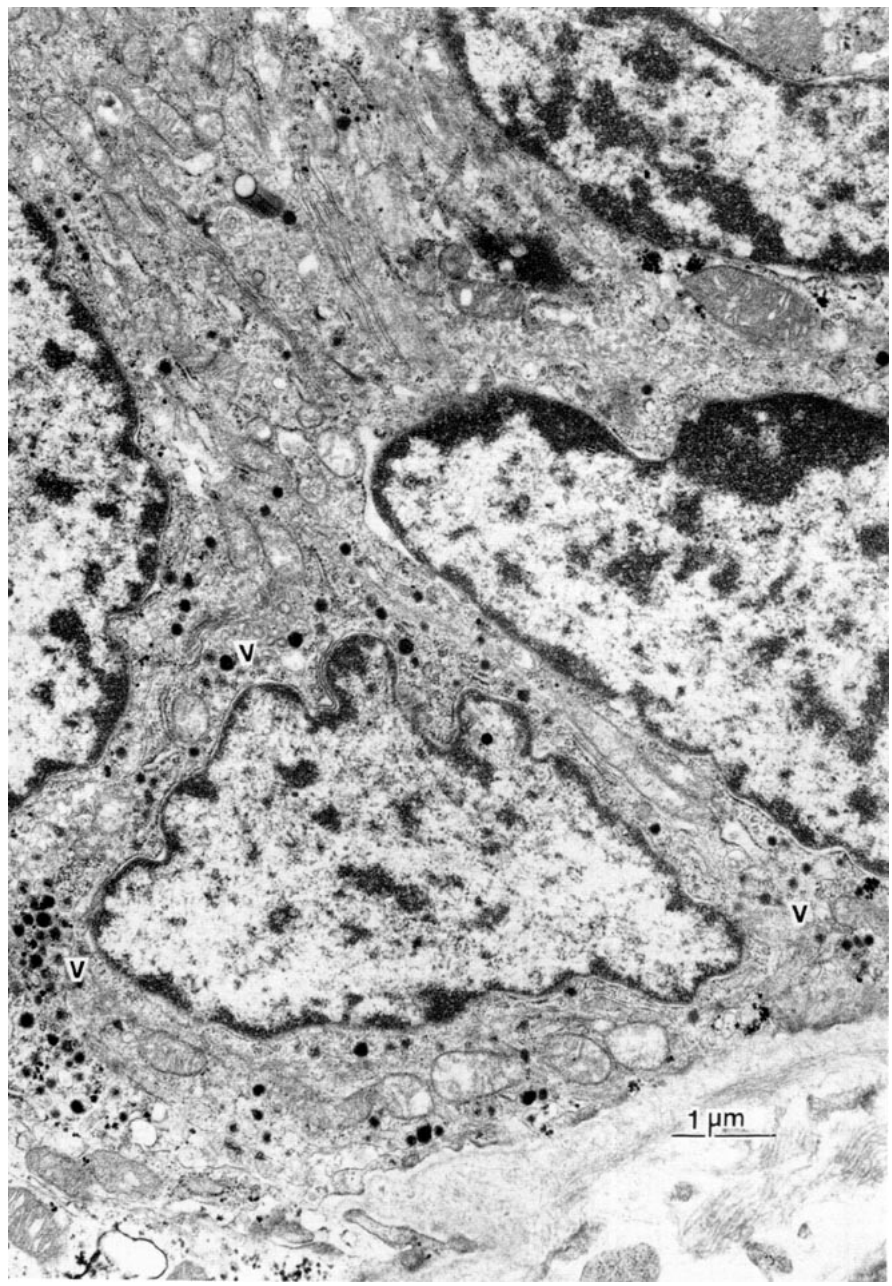

FIGURE 9. Portion of a neuroepithelial body in neonatal human airway epithelium showing three cells with dense-core vesicles (v). TEM. ×14,000. Contributed by Dr. E. Cutz.

cent products with substances containing primary amino groups (*110*). This compound gives strong and selective fluorescence of various "polypeptide hormone-secreting cells" when tissue specimens are fixed in formaldehyde vapors (*111*). The fluorogenic component was thought to be associated with the secretory granules. Lauweryns and Liebens (*30*) performed a microspectrographic analysis of formaldehyde-fixed, neonatal rabbit lung NEBs treated with fluorescamine and proposed that polypeptides, not serotonin, were responsible for the emitted fluorescence. The potential of this compound as a cytochemical reagent for identifying lung neuroendocrine cells should be further explored.

Sorokin and Hoyt (*22*) found that periodic acid Schiff (PAS)–lead-hematoxylin was suitable for mapping "small-granule endocrine cell populations" in the lungs of various species; both K cells and NEBs were stained. Using a monoamine fluorescence technique on plastic sections of lungs from control and 5-HTP-pretreated animals prior to staining, they showed that the fluores-

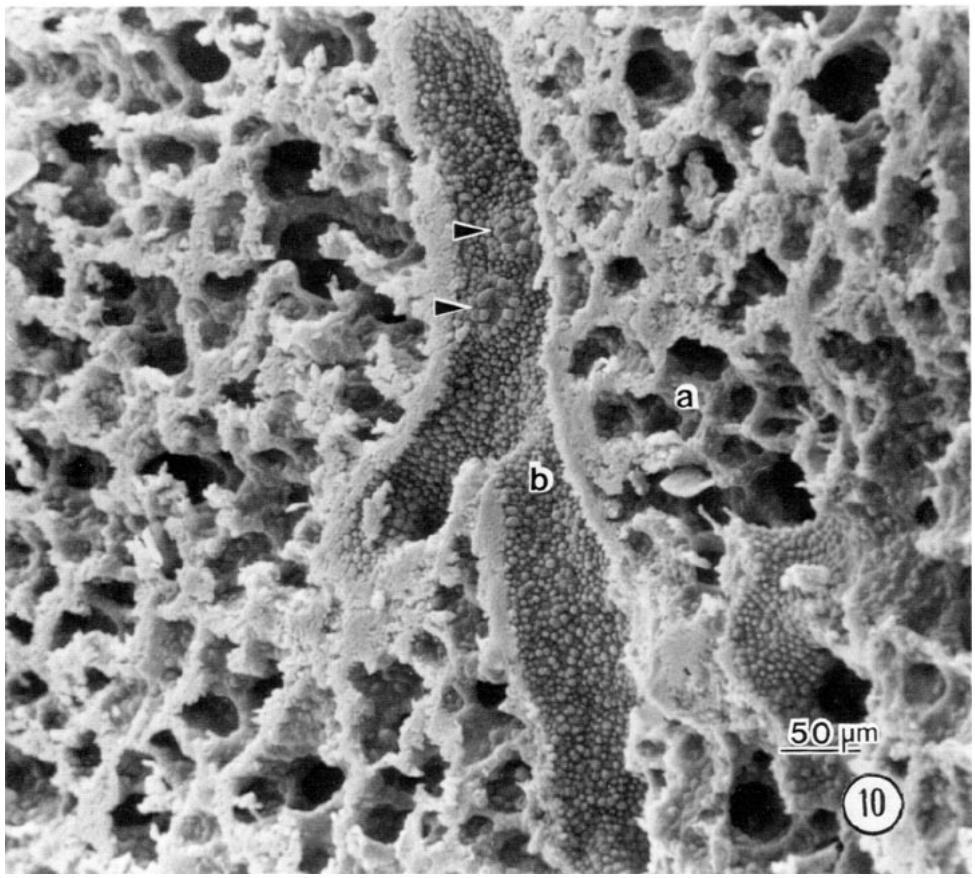

FIGURE 10. Scanning electron microscopy view of the luminal surface of a terminal bronchiole and alveolar spaces in rabbit fetal lung. Two neuroepithelial bodies (arrows) located near a bronchiolar branching. The airway surface is nonciliated and composed of dome-shaped cells. SEM. ×200. Reproduced from Cutz and Sonstegard (*24*).

cent epithelial cells were identical to those stained by PAS–lead-hematoxylin.

Neuron-specific enolase, a brain isoenzyme of the glycolytic enzyme, has also been shown to be a marker for peripheral and central neuroendocrine cells (*87*). Immunolocalization of this enzyme in human fetal bronchial epithelium was recently investigated by Polak and Bloom (*7*), who found neuron-specific enolase in cells containing both bombesin and serotonin, serotonin alone, and in a separate population of epithelial cells. These results would seem to endorse the presence of multiple populations of cells with neural (neuroendocrine) characteristics in lung epithelium. This immunocytochemical method should be especially useful to investigators needing a marker not associated with secretory granules.

Distribution and Quantitation

As has been indicated previously, K cells are found in the trachea and intrapulmonary airways of the human and other animal species, in both fetuses and adults (*92*,*112*). There are few comprehensive or systematic studies, however, of the distribution or quantitation of K cells, especially with regard to different airways. On the other hand, NEBs, regardless of species, are found only in the epithelium of the intrapulmonary airways and often at or near bronchiolar bifurcations. As a general class, lung neuroendocrine cells are considered rare (*102*).

Tateishi (*15*) examined sections of lungs from Japanese adults and reported that argyrophilic cells in "small numbers" were scattered among the epithelial cells that line the bronchi, as well as bronchioles. The frequency of these cells tended to increase as the caliber of bronchus decreased and this appeared to be independent of age. Argyrophilic cells were sparse, however, in the terminal bronchioles and among epithelial cells that lined the ducts and acini of bronchial glands. The frequency of argyrophilic cells increased in regions of the bronchioles exhibiting goblet cell hyperplasia. In contrast, few argyrophilic cells were detected in areas of squamous metaplasia. As reported by Cutz and Orange (*102*), the quantitation of APUD cells by silver impreg-

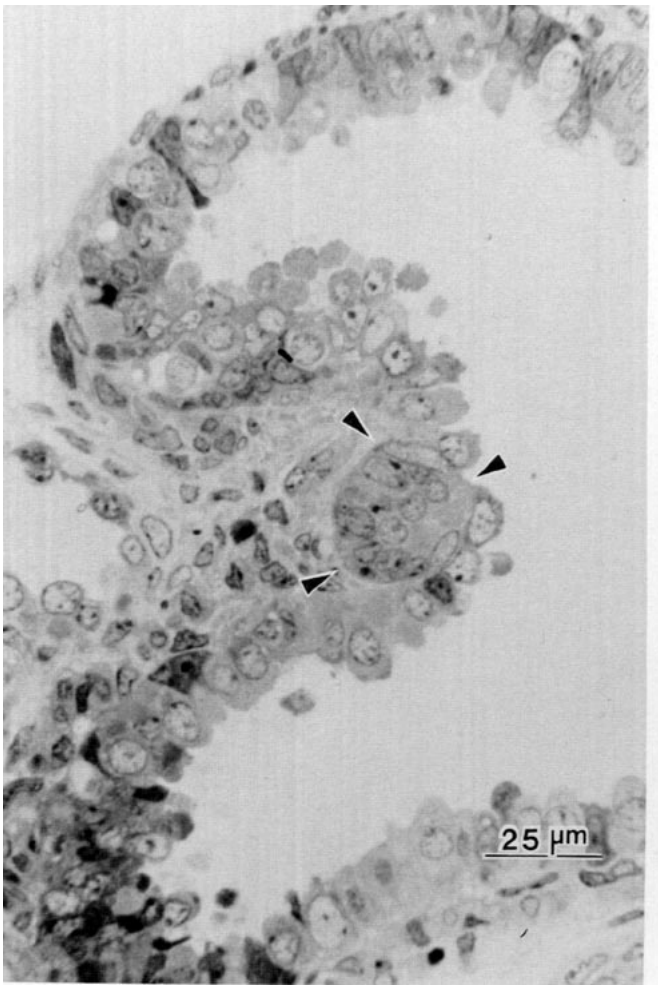

FIGURE 11. Light micrograph revealing the ovoid corpuscular appearance and columnar cells of a neuroepithelial body (arrows) at an airway bifurcation in near-term fetal rabbit lung. 1-μm plastic section. Toluidine blue. ×640.

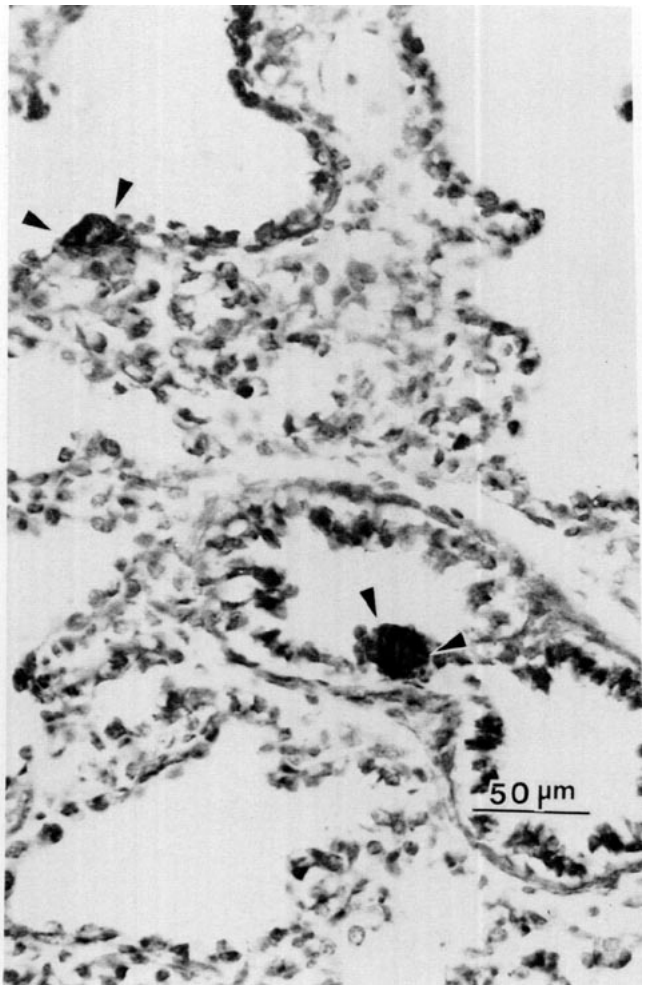

FIGURE 12. Neuroepithelial bodies composed of several argyrophilic cells (arrows) in a paraffin section of whole, 29-day fetal rabbit lung fixed in paraformaldehyde–glutaraldehyde–picric acid. Grimelius stain. ×320.

nation in human fetal lungs from 8 to 20 weeks gestation revealed that the numbers of these cells increased from an average of 9/mm^2 of whole lung section at 8 weeks gestation to 20/mm^2 during the early canalicular period (10–12 weeks). The APUD cells were also more frequent in proximal, more developed bronchial tubes as compared to terminal buds. According to these workers, later in development (14 weeks gestation) there was a decline in the number of APUD cells per square millimeter of lung tissue, but the number of APUD cells per airway remained unchanged, suggesting "dilution" of APUD cells during growth and differentiation of lung. Lauweryns and Peuskens (*10*) examined the occurrence of argyrophilic cells in the lungs of premature and newborn infants and found the cells present in major bronchi, smaller bronchi and bronchioles, and distal portions of respiratory bronchioles. The approximate frequencies of airways exhibiting argyrophilic cells for each of the three divisions was 37, 22, and 56%, respectively. Since this study predated the reported recognition of NEBs, the quantitative data may include these organoids, as well as the K cells.

Palisano and Kleinerman (*51*) quantitated APUD cells (solitary K cells) and NEBs in midcoronal sections of lungs of adult hamsters pretreated with 5-HTP and L-DOPA and found that formaldehyde-induced fluorescence gave greater numbers of both categories of cells than did silver staining. Individual APUD cells were present at a frequency of 0.31×10^{-1} cell/mm (airway perimeter) in bronchi and $0.2–0.24 \times 10^{-1}$ cell/mm in bronchioles. NEBs were found at a similar frequency in bronchi (0.18×10^{-1}/mm), large bronchioles (0.28×10^{-1}/mm), and small bronchioles (0.19×10^{-1}/mm). Moosavi et al. (*44*) quantitated the number of argyro-

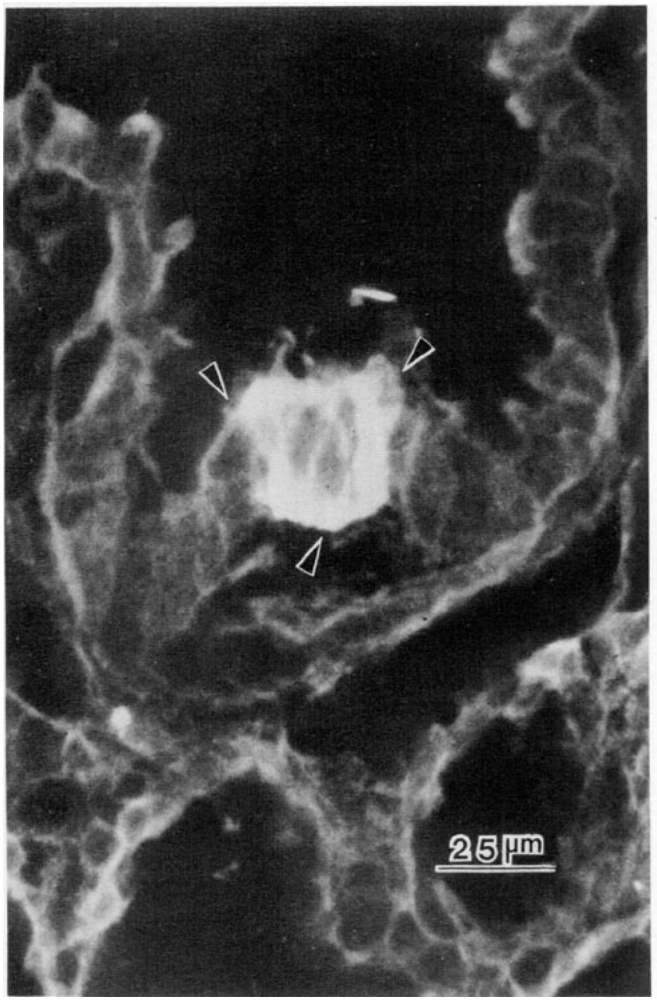

FIGURE 13. Neuroepithelial body (arrows) in airway mucosa of a 29 day fetal rabbit lung revealed by formaldehyde-induced fluorescence. Paraffin section. FIF ×640.

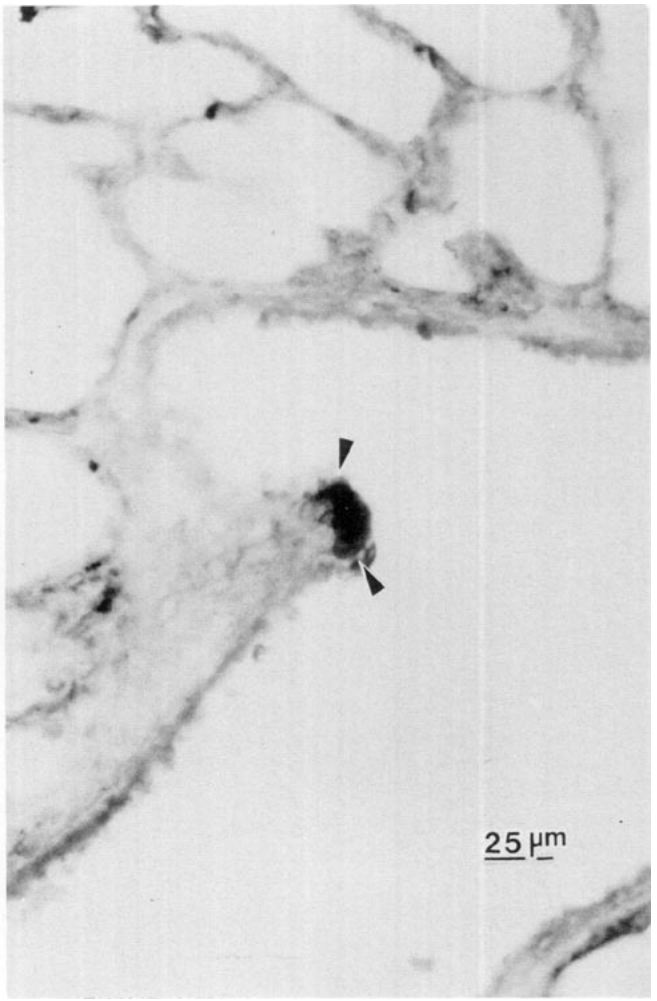

FIGURE 14. Neuroepithelial body (arrows) at an airway bifurcation demonstrated by peroxidase-antiperoxidase (PAP) immunostaining for serotonin, in 29-day fetal rabbit lung. Paraffin section. Toluidine blue counterstain. ×400.

philic (Feyrter) cells/cm (airway perimeter) in the bronchial epithelium of 4 to 31-day-old rats and reported that the cells increased from 32–59/cm to 70/cm between day 4 and day 7 of life and decreased to 16/cm by day 31. Palisano and Kleinerman (*51*), however, could not confirm these numbers in young rats and found about one-tenth of the argyrophilic cells reported by Moosavi et al. (*44*) close to the number the former authors found for the adult hamster.

Recently, Hoyt et al. (*44*) reported detailed quantitative and distributional analyses of APUD cells in the infracardiac lobe of a hamster lung using glycol methacrylate, PAS–lead–hematoxylin-stained sections. "Small-granule endocrine" cells were found in the epithelium lining lobar bronchi and bronchioles and at the bronchiole–alveolar portals. The cells occurred singly and in groups of 2 to 56 cells at a mean density of 6 solitary cells and 10 cell clusters (NEBs)/mm of airway length. Moreover, with the PAS–lead–hematoxylin stain these workers were able to identify five APUD cell types. Furthermore, the component cells of NEBs were not homogeneously stained and revealed multiple cell types by this staining method. It is important to caution that these so-called "different" cell types may represent various stages in the life cycle of a single population of neuroendocrine cells.

Dey et al. (*93*) examined the distribution of "serotonin-containing" cells in tracheal epithelium of the adult rabbit by formaldehyde-induced fluorescence. No significant differences in the frequency of fluorescent cells were found in the dorsal region of the cranial, middle, and caudal segments of the trachea. The dorsal mucosa, however, contained significantly fewer fluorescent cells than that of the ventral. They also reported significantly more fluorescent cells in the ventral, cranial segment than in other regions; this segment contained a mean of 15 cells/15-µm section. In one way, this report appears to confirm the findings of Ericson et al. (*58*), who

demonstrated numerous fluorescent cells in the mouse larynx and the region of the trachea just beneath the cricoid; in the more caudal aspect of the trachea and in the main bronchi, fluorescent cells were few in number. Kirkeby and Romert (*39*) identified argyrophilic epithelial cells in the larynx of the guinea pig. The cricoid epithelium of the guinea pig larynx was recently quantitated for K cells by electron microscopy in our laboratory (*96*). It was found that small granule cells were evenly distributed and present, on the basis of ultrastructure, as a homogeneous population constituting 5% of the epithelial cells in newborn and young adult animals. This frequency decreased gradually from cranial to the caudal segments of the trachea. Just above the carina, small-granule cells constituted only 1 to 2% of the epithelial cells. Very few of these cells were identified in the main bronchi. Kleinerman et al. (*113*) quantitated rat airway argyrophilic cells/100 epithelial cells and reported values of 0.13 in the trachea, 0.03 in the large airways, and 0.02 in the small airways. These studies indicate that K cells may not necessarily be a rare cell population in some regions of the airways, and confirms that they may be present at higher frequencies in the larynx and cranial aspect of the trachea than in more distal parts of the respiratory tract.

Function

K Cells

The paucity of K cells and their diffuse distribution have been major factors in limiting investigations primarily to microscopy and have made it difficult to examine functional aspects of these cells. Despite this, it can be speculated from their histochemical characteristics and ultrastructure and by analogy with similar neuroendocrine cell types in other tissues that K cells may elaborate a peptide hormone(s) that influences nearby or distant cells. Appreciation of known endocrine or neuroendocrine concepts may help to identify the function of the K cell. The distance a hormone must travel to elicit a biological response is one factor to consider. K cells may elaborate a bloodborne hormone which regulates cells of the lung or some other organ. Hormones from the pituitary act on organs at a considerable distance, e.g., corticotropin on the adrenal gland. If this were also the case for K cells, they would function as true endocrine cells. On the other hand, the distribution of these cells in the airways suggests that K cells may also have a local, or paracrine function, whereby other epithelial cells (basal cells or mucous cells) or submucosal cells (smooth muscle or nerves), may be the specific target. Some peptides in the intestine, for instance, are considered to integrate cell and tissue functions in close proximity to the locus of their release (*114,115*). Others, such as gastrin, produced by cells in the antrum, become bloodborne and can exert effects at more distant sites within the same organ.

Another important concept is the effective concentration of mediator or hormone required to evoke a response in the target cells or tissues that possess the complimentary membrane receptors. The affinity constants of polypeptide hormones for receptors are usually very high, which is consistent with the low levels of bloodborne hormones required to produce effects *in vivo* (*116*). Within the confines of a putative paracrine function for K cells, it is conceivable that enough hormone may be secreted to affect nearby target cells. Proteases, nonspecific binding to cell surfaces or circulating proteins, and dilution all can readily reduce bioavailability of a hormone. These factors are less likely to influence paracrine secretions to the same extent that they influence bloodborne hormones. Therefore, the cumulative hormone output of K cells need not be within the magnitude exhibited by classical endocrine glands. Of course, if the target is in intimate contact with the K cell, as through synaptic junctions or gap junctions, relatively high concentrations of hormone could be attained and exclude the need for high affinity binding sites on the post-junctional membrane.

The widespread distribution of solitary K cells suggests that these cells, as a homogeneous or heterogeneous class, may regulate such functions as the secretion of mucus (*15*), airway muscle tone (*10,112*), integration of ventilatory activities in lobules and lobes (*9,117*), pulmonary perfusion (*9,117*), cell differentiation (*2*), cell permeability, or ciliary motility. The appearance of neurosecretorylike granules in mucous cells of human segmental bronchi led Terzakis et al. (*16*) to postulate that neuroendocrine cells could serve as a primary or accessory source of mucous cells under ordinary or pathologic conditions. Tateishi (*15*) found "argyrophilic cells" in areas of mucous cell hyperplasia in human lung and proposed that these cells may secrete a humoral substance responsible for the production of mucus in other epithelial cells.

Neuroepithelial Bodies

As with solitary K cells, investigations of NEBs have been limited to morphological and cytochemical studies and have not determined what specific functions these structures serve in man or animals. Correlation of anatomical, ultrastructural, and cytochemical data with limited physiological studies have led investigators to speculate that NEBs function as intrapulmonary chemoreceptors. Even before the discovery of NEBs in various species, workers postulated the existence of intrapulmonary chemoreceptors that might respond to changes in the gas composition. Comroe (*118*), in a portentous comment, wrote, "chemical receptors could be located in the pulmonary alveoli and sense the composition of alveolar gas; these could determine whether changes in total ventilation changed alveolar ventilation by the proper amount." Using direct vagal recordings, Fedde and Peterson (*62*) established that avian intrapulmonary receptors increased their dis-

charge as intrapulmonary CO_2 decreased. In contrast to mammals, these receptors were not sensitive to intrapulmonary pressure, hyperoxia or hypoxia. Barnas et al. (*70*) later ruled out the possibility that these receptors were mechanoreceptors, since consistent changes in the parabronchial lumina were not observed as a result of changes in intrapulmonary CO_2 concentration. Cook and King (*61*) and later King et al. (*63*) extensively examined sensory nerve endings in avian lung. These workers identified an afferent synaptic complex in the primary bronchus associated with a specialized cell whose cytoplasm contained dense-core vesicles similar to those in the glomus cell of the carotid body and in mammalian lung NEB cells. These specialized avian lung cells occurred in small groups and were postulated to be a neurite-receptor complex, possibly the proposed CO_2 chemoreceptor. Later studies by Wasano and Yamamoto (*66*) also demonstrated intraepithelial nerve endings which formed synapselike junctions on granule-containing epithelial cells in intrapulmonary airways of avian lung. These authors proposed that such nerve-cell complexes and similar structures in the lungs of other species may function as chemoreceptors.

Further evidence that NEBs may function as chemoreceptors was presented by Lauweryns et al. (*35*) in a comprehensive study of neonatal rabbit NEBs under various conditions. Both hypoxia and hypercapnia decreased amine cytofluorescence with an apparent corresponding increase in exocytosis of dense-core vesicles from the basal cytoplasm of component neuroendocrine cells. They reported that hypercapnia produced a fragmentation of the vesicles with the formation of pale, granular central cores, which had the appearance of type 2 dense-core vesicles. It was later concluded from cross-circulation studies that NEBs react directly to the hypoxia of inhaled air and not to hypoxemic conditions of the pulmonary blood (*36*). Exocytosis of dense-core vesicles has also been observed in the glomus cells of the carotid body under conditions of hypoxia or hypercapnea; these morphological changes appear to be organ-specific, since acute hypoxia did not produce such alterations in other APUD-type cells of the rat adrenal medulla (*119*). The carotid bodies of inhabitants of the Andes are 3 to 6 times larger than those living at sea level (*120*). In an analogous finding, Taylor (*46*) reported that the number of solitary argyrophilic cells and groups of argyrophilic cells in lung was significantly greater in rabbits raised in the Andes than their counterparts at sea level. Thus, acute and chronic adaptive responses of NEBs and carotid bodies under hypoxic conditions may have morphological and functional features in common. Details of the responses of lung neuroendocrine cells to hypoxia are discussed in the "Pathobiology" section of this review.

Treatment of neonatal rabbits with reserpine resulted in a depletion of serotonin in NEB cells, as determined by microspectrography, with an accompanying loss of dense-core vesicles, altering the morphology of type 1 vesicles to that of type 2 (*35*). The ultrastructural effect of reserpine on NEB dense-core vesicles was confirmed by Sonstegard et al. (*31*) using 7-day lung explants from near-term fetuses. Similar reserpine-induced effects have been reported for other amine-storing, or APUD-type cells, such as in the carotid body (*121*) and adrenal medulla (*122*). Since the microspectrographic emission spectrum from formaldehyde-induced fluorescence of NEBs corresponds to that of serotonin, Lauweryns and his colleagues (*35*) postulated that this indolealkylamine is stored in the NEB-cell type 1 dense-core vesicles and may be responsible for hypoxic pulmonary vasoconstriction. Serotonin can produce constriction of pulmonary blood vessels (*42,123,124*) and it is conceivable, although unlikely, that hypoxia-induced degranulation of a NEB can release enough (*42*) of this amine into the local pulmonary vascular bed to modulate lung perfusion and function. Accordingly, this notion has been used to explain the chronic pulmonary vasoconstriction in fetal lung, the rapid pulmonary vasodilation at birth, and pulmonary hypoperfusion associated with the respiratory distress syndrome (*34*). Said et al. (*125*) proposed that alveolar hypoxia may provoke the synthesis and release of prostaglandins and additional mediators, such as lung peptides, which add to the pulmonary vascular response to hypoxia.

The strategic location of NEBs with their apices exposed to the lumen at airway bifurcations and the proposed afferent-efferent innervation make it reasonable to speculate that these structures provide an intrapulmonary chemoreceptor system that finely adjusts the balance between ventilation and perfusion. The numerous correlates that exist between the pulmonary NEB and the carotid body encourage investigation of a chemoreceptor function for pulmonary NEBs. Isolated, intact mammalian NEBs (*101*) should provide an appropriate experimental system to identify the amines and peptides in these structures and learn more about their biochemical and physiological functions. Again, strong correlates are likely to be found with the carotid body.

Peptides

Cells of the APUD series and peptidergic neurons represent an anatomically diverse and extensive system of neurosecretory cells that release various peptides which regulate a number of physiological events in the central nervous system and peripheral organ systems (*126–129*). The fact that identical peptides may be secreted from both epithelial cells and neurons indicates that some peptides may have more than one regulatory role. More than 20 different peptide hormones have been identified in the brain and gastrointestinal tract. Armed with specific antisera to these peptide hormones and immunocytochemical techniques, investigators have examined the morphology of the peptidergic cells and the distribution of biologically active peptides in organs of different species (*130*). Knowledge of the existence of a nonadrenergic, noncholinergic nervous system in the

lungs of mammals has arisen from studies (*99,131–135*) that showed a relaxant effect to electrical nerve or field stimulation of isolated smooth muscle of conducting airways. There is now evidence, as indicated below, that neuropeptides may be functional in the lung and regulate smooth muscle contraction and other nonrespiratory events.

The peptides that have been identified in the lung are listed in Table 2 (*136–148*). The pulmonary and gastrointestinal systems both exhibit immunoreactivity to peptides in nerves, submucosal glands and epithelial cells. However, it is important to consider that the qualitative identification of most of the lung peptides in tissue sections or in extracts has relied on cross-immunoreactivity with antisera to known polypeptides. Therefore, careful consideration is necessary when using immunocytochemical techniques or radioimmunoassays, since these methods may yield false positives or negatives (*37,149–152*). It is imperative that investigators employ various physicochemical procedures and bioassays, if possible, to verify the identity of unknown immunoreactive material with a known peptide.

Pathobiology

Ectopic or inappropriate hormone production by human lung tumors has stimulated interest in lung neuroendocrine cells. Recent reviews (*153–155*) on clinical aspects of ectopic hormone formation and the variety of polypeptide hormones involved preclude the need to cover this topic. Invariably, clinically manifested endocrine abnormalities in patients with lung tumors are associated with small cell carcinoma (*156*). For some time now, the lung K cell has been considered the cell of origin for lung small cell carcinomas and bronchial carcinoid tumors (*14,157–162*). This concept was based on the fact that these tumors revealed ultrastructural features, i.e., the presence of dense-core vesicles, and cytochemical properties which are similar to pulmonary K cells (*14,112,157,159,163,164*). An "endocrine cell hypothesis" was formulated to explain ectopic hormone production in various organs (*153,154,157,165,166*). According to this hypothesis, tumors which synthesize ectopic hormones are derived from endocrine cells which previously had made a different hormone.

There is no experimental evidence demonstrating that endocrinelike tumors in nonendocrine organs, such as lung, originate from neuroendocrine cells. In fact, some studies propose an alternative progenitor cell–tumor relationship for small cell carcinomas and bronchial carcinoid tumors in lung. These views are discussed by Baylin and Mendelsohn (*157*) and McDowell et al. (*161*), who concluded that APUD characteristics in tumors do not necessarily reflect origin from a progeni-

Table 2. Immunoreactivity to known peptides in mammalian lung.

Immunoactive peptide	Species	Location	Method of detection	Reference
Vasoactive intestinal polypeptide (VIP)	Porcine, canine, feline, human, rabbit	Tissue extracts	Bioassay, RIA[a]	(*136*)
	Feline, rabbit, guinea pig	Nerves around seromucous glands, blood vessels, smooth muscle in tracheobronchial wall	IMCC[b]	(*137*)
	Feline	Nerves around gland acini and arterioles	IMCC	(*138*)
	Guinea pig	Medium from isolated trachea after electrical field stimulation	Bioassay, RIA	(*139*)
Substance P	Canine, murine	Tissue extracts	RIA	(*140*)
	Guinea pig	Nerve fibers in smooth muscles and in submucosal connective tissue of trachea and bronchi	RIA; IMCC	(*141*)
		Nerve fibers in walls of tracheobronchial arteries and veins	IMCC	(*142*)
	Murine	Nerve fibers associated with blood vessels and airways in all regions of the lung	IMCC	(*143*)
	Feline	Nerve endings around blood vessels and in epithelium	IMCC	(*138*)
Bombesin	Human fetus and neonate	Tissue extracts; epithelial cells	RIA; IMCC	(*144*)
	Human fetus, neonate and adult	Epithelial cells	IMCC	(*145*)
Calcitonin	Simian	Tissue extracts	RIA	(*146*)
	Human neonate	Bronchial and bronchiolar epithelium	IMCC	(*147*)
	Human fetus, neonate and adult	Epithelial cells	IMCC	(*145*)
Physalaemin	Porcine, rabbit	Tissue extracts	RIA	(*148*)

[a] Radioimmunoassay.
[b] Immunocytochemistry.

tor cell, but rather, that these characteristics arise as a consequence of a particular route of differentiation. In this regard, the formation of lung tumors with endocrinelike properties would be determined by the capacity of a pluripotent nonendocrine cell to differentiate into cells with APUD characteristics. This differentiation may be influenced by a selection process which favors endocrine properties or an increased proliferative capacity, or both. Ectopic production of mitogenic peptides, such as vasopressin, may be linked to the rapid proliferation properties exhibited by human small cell carcinomas (*167*). Thus, the relatively high frequency of lung small cell carcinomas, about 20 to 30% of human lung tumors (*168*), can be explained despite the fact that APUD cells occur in low numbers in the pulmonary epithelium.

Some reports in the past decade have indicated that the number and morphology of K cells or NEBs is affected by environmental factors which include carcinogenic compounds (*54,169*) particulates (*170,171*), noxious gases (*17,51*), and ventilatory gases with altered composition (*33,44,52*). Reznik-Schüller examined the bronchial epithelium of hamsters treated subcutaneously with diethylnitrosamine (*52,54*), dibutylnitrosamine (*52*), or *N*-nitrosomorpholine (*172*). After treatment for three weeks, a majority of the animals exhibited hyperplasia of "APUD-type cells" consisting of clusters of 8 to 40 cells in segmental bronchi and bronchioles; these cells contained numerous dense-core vesicles. Granules were reported to decrease in number with an accompanying increase in cytoplasmic filaments after longer periods of treatment. It was postulated from these observations that the initial phase of APUD cell proliferation progressed to squamous metaplasia (*54,172*). No lung tumors developed in hamsters treated up to 20 weeks (*52*). Identification of hyperplastic areas with dense-core vesicles was not observed in hamsters during the initial stages of benzo(a)pyrene-induced pulmonary carcinogenesis (*173,174*). These studies may indicate that the carcinogenic nitrosamines preferentially stimulate growth of lung neuroendocrine cells. Becci et al. (*169*) reported that of the 51 hamsters which developed lung tumors following intratracheal instillations of benzo(a)pyrene-Fe_2O_3, one had a metastasizing tumor that morphologically resembled a human bronchial carcinoid tumor. Cells from the hamster lung tumor contained dense-core vesicles, 110 to 220 nm in diameter. Recently, Linnoila et al. (*50*) reported that diethylnitrosamine-treated hamsters revealed a significant increase in the number of argyrophilic cells of NEBs (Fig. 15); the number of solitary argyrophilic cells remained unaltered. In order to examine whether treated neuroendocrine cells showed enhanced survival *in vitro*, lung cells were enzymatically dissociated and placed in culture. After 7 days, many of the cells derived from hamsters treated for 5 to 8 weeks demonstrated argyrophilia, dense-core vesicles and corticotropinlike immunoreactivity. These workers suggested that the neuroendocrinelike cells in the cultures were derived from hyperplastic neuroendocrine cells in lungs of treated animals. Since the "APUD cell" hyperplasia reported by Reznik-Schüller (*52*) in treated animals was confined to segmental bronchi and bronchioles, where NEBs are generally located, it is conceivable that these organoids, or preferably their progenitors, are the primary sites interacting with the carcinogen to produce the hyperplasia of neuroendocrine cells.

Abnormal proliferation of lung neuroendocrine cells also occurred in rats exposed for 6 months to 2 years to chrysotile or crocidolite asbestos (*170*). Seven of 26 exposed animals had groups of endocrinelike cells containing cytoplasmic dense-core vesicles (100–180 nm diameter) in respiratory bronchioles and alveoli. Castleman and co-workers (*17*) reported an increased number of granulated cells in respiratory bronchioles of rhesus monkeys exposed to ozone; these cells increased from approximately 0.5% of the epithelial cells in unexposed animals to almost 9% in animals exposed for 26 or 36 hr. Their presence in stratified clusters of nonciliated bronchiolar cells led these workers to propose that K cells were dividing in response to ozone exposure, although K cells were not identified in mitosis by electron microscopy or autoradiography.

Present data suggest that lung neuroendocrine cells divide infrequently or do not proliferate under normal conditions, making the neuroendocrine cell phenotype an unlikely source of hyperplastic cells (*27*). Early events which lead either to an increase in lung neuroendocrine cells or formation of endocrinelike tumors in man are probably initiated in nonneuroendocrine cells, as suggested by others (*157,161*). There is both direct and indirect evidence which indicate that lung neuroendocrine cells differentiate from cells that do not have APUD characteristics. Tsubouchi and Leblond (*175*) examined enteroendocrine cell turnover and differentiation in mouse small intestine and reported that these cells have their origin in a precursor cell common to vacuolated columnar and mucous cells. The turnover times and migration rate of these cells varied but they all were formed by differentiation from an endodermal crypt-base columnar cell. This concept has replaced earlier, more tenuous views, that considered, for instance, that endocrine argentaffin cells were a phase in the differentiation cycle of mucous cells (*176*).

In a cytokinetic study of rat tongue taste buds, which have some structural similarities to pulmonary NEBs, Biedler and Smallman (*177*) investigated cell renewal within this organoid with [^{3}H]-thymidine. they concluded that the epithelial cells immediately adjacent to taste buds divide and that some of the daughter cells enter the taste bud and slowly move toward the center. The rate of entry of the labeled cell into the taste bud was about one every 10 hr. In a similar study, Hernandez-Vasquez et al. (*27*) examined the incorporation of [^{3}H]-thymidine into fetal and neonatal rabbit NEBs and could not find any label in these organoids up to 24 hr after injection of the isotope. In addition, no mitotic figures were found in any of the 1000 NEBs

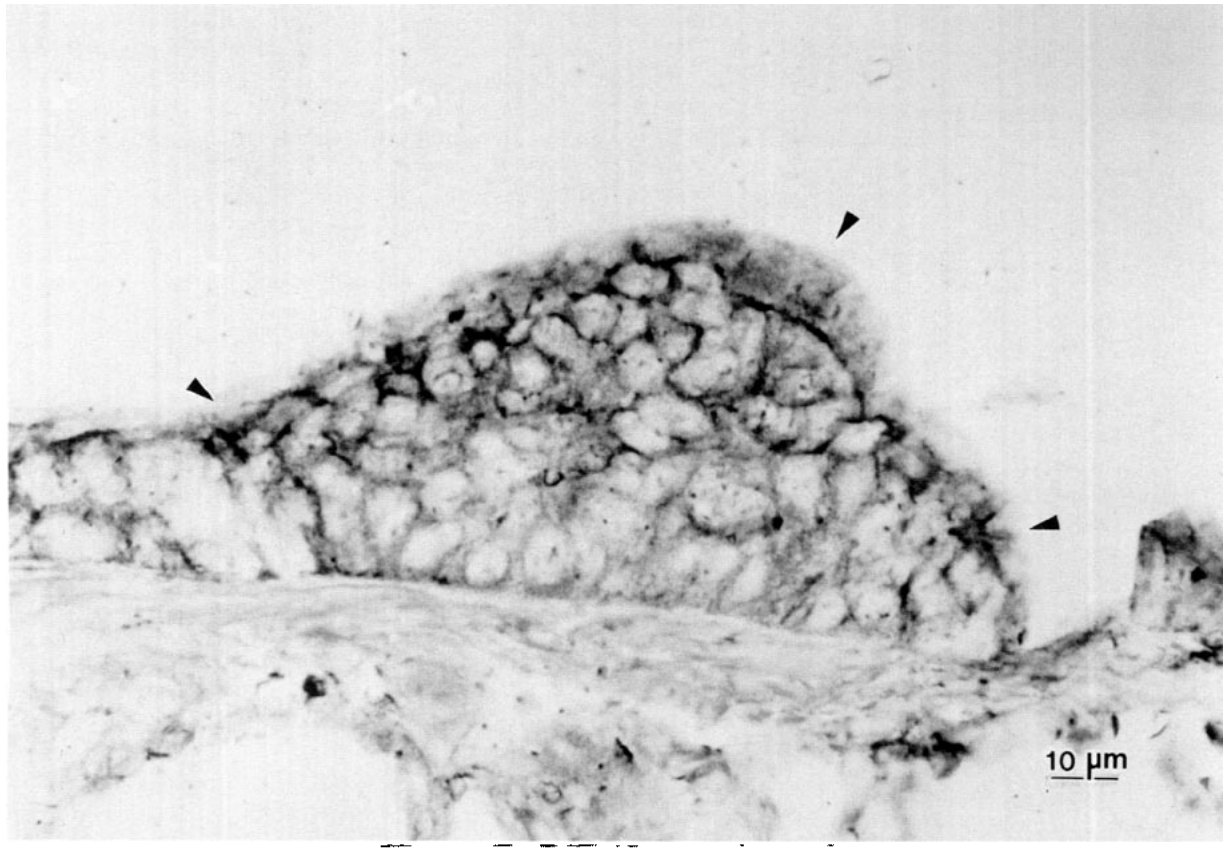

FIGURE 15. An apparent hyperplastic group of cells (arrows) similar to a neuroepithelial body in intrapulmonary airway of adult hamster treated subcutaneously, twice weekly, for 8 weeks with 3 mg diethylnitrosamine. Paraffin section; Grimelius stain. ×1000. Preparation by Dr. R. I. Linnoila.

examined. This finding and those of Sorokin et al. (*32*) and Sonstegard et al. (*101*) suggest that NEBs are well differentiated and originate from other types of epithelial cells in the airways.

By aligning these findings with some concepts of chemical carcinogenesis, we may offer a rationale as to how a carcinogen such as diethylnitrosamine can induce hyperplasia of lung neuroendocrine cells through interaction with Clara cells. First, as stated earlier, Clara cells or morphologically similar relatives have been reported to be intimately associated with NEBs (*24,56*). Second, labeling studies with [^{3}H]-thymidine support that Clara cells have the capacity to divide and may have a progenitor role (*178*). Third, studies of the distribution of diethylnitrosamine in the lung indicated that in hamster lobar and segmental bronchi and bronchioles, this compound showed preferential localization in Clara cells (*179*) and can affect the morphology of these cells (*53*). Finally, Clara cells can metabolize xenobiotics through a monooxygenase system (*180*), which has been shown to convert noncarcinogenic compounds to carcinogenic ones (*181*). Details on this aspect of the Clara cell are given elsewhere in this issue (*182*).

One consequence of the interaction of diethylnitrosamine with Clara cells may be the stimulation of the rate of normal differentiation of these cells to neuroendocrine cells of NEBs. Alternately, the expression of the neuroendocrine phenotype may be the consequence of Clara cells (modified by diethylnitrosamine) selecting, albeit preferentially, an abnormal route of differentiation. It is conceivable that the initiation of carcinogenesis in nonneuroendocrine cells by chemical compounds, such as nitrosamines, can lead progressively to the formation of neuroendocrine cell hyperplasia (*50*), pulmonary tumorlets (*183,184*), bronchial carcinoid tumors, and small cell carcinomas (*14,159,160*).

Sorokin et al. (*185*) analyzed sections of a human bronchial carcinoid tumor by PAS–lead–hematoxylin and by conjunctive staining, whereby in tissue previously reacted for argyrophilia an investigator can examine sequentially the fluorescence for serotonin, PAS alone, and PAS–lead–hematoxylin in a single section. By this method, nine different endocrine cell "signatures" were identified in the tumor. The results from electron microscopy (*186*) confirmed the heterogeneity of component endocrinelike cells; at least three general classes of cells in the tumor were determined on the basis of differences in granule ultrastructure. An unusual obser-

vation was the presence of numerous tumorlets in the bronchial epithelium overlying the tumor with a few of the tumorlets containing cells with both mucous and dense-core granules (*185,186*). It was also noted that the frequency of the tumorlets exceeded that for normal small granule endocrine cells in the human bronchial epithelium. Taken together, the data led these workers to propose that the carcinoid tumor originated from non-APUD stem cells. They viewed that "in normal bronchial epithelium a common cell gives rise to a small pool of APUD cell precursors and a much larger self-renewing pool of nonendocrine cell precursors, from which basal, mucous, and ciliated cells ultimately are derived."

In addition to the studies by Lauweryns et al. (*34–36,187*), other investigators have examined quantitative and qualitative aspects of short- and long-term hypoxia on lung neuroendocrine cells. Moosavi et al. (*44*) reported ultrastructural changes in dense-core vesicles of "Feyrter," or K cells, in hypoxic rats. These workers concluded that the ultrastructural changes occurring in the K cells were identical to those found in carotid body glomus cells of animals exposed to high-altitude hypoxia (*120*). No histological changes were observed for lung argyrophilic cells. Later studies by Hernandez-Vasquez et al. (*26,188*) examined the quantitative changes of argyrophilic K cells in the lungs of fetal and neonatal rabbits under normoxia and short-term hypoxia. Hypoxic animals apparently had significantly lower numbers of K cells and NEBs when compared to normoxic animals of the same age group. It is important to consider again that quantitative results may be misleading if the staining properties of K cells or NEBs are affected by experimental conditions. A case in point is the study by Keith et al. (*33*), who showed that the comparison of serotonin emission intensity in NEBs of acutely hypoxic neonatal rabbits and normoxic controls revealed significantly less fluorescence in hypoxic animals. Neuroendocrine cell numbers, however, as shown by the Grimelius silver stain, did not change. In another study, Palisano and Kleinerman (*51*) used the Falck-Hillarp (FIF) technique to quantitate K cells and NEBs in normal and NO_2-exposed animals after priming hamsters with 5-HTP and L-DOPA. The number of K cells decreased to approximately 25% of control levels after 24 hr of exposure to 30 to 40 ppm of NO_2; after 28 days of exposure the K cells decreased to 50% of control levels. The number of NEBs decreased transiently after 24 hr of NO_2 but returned to control levels by day 14. Neuron-specific enolase (*7,87*) may be the more reliable means of identifying lung neuroendocrine cells in treated animals. It is likely that cells will retain this isoenzyme even though degranulation or loss of biogenic amines has occurred following exposure to an oxidant gas.

Concluding Comments

It is clear from the numerous reports that the tracheobronchial epithelium of all mammals examined, as late-stage fetuses or adults, contains neuroendocrine cells. The paucity of these cells, either as solitary K cells or component cells of the neuroepithelial body, should not distract the investigation of their potential vital regulatory roles. To this end, the successful isolation and purification of lung neuroendocrine cells should allow identification of their bioactive peptides. The type and age of animal and region of the airways to apply cell dissociation methods are critical factors in obtaining significant numbers of neuroendocrine cells for analyses. Fluorescein-conjugated antibodies to neuroendocrine cell-specific proteins may enable investigators to employ flow cytofluorometry in the isolation and characterization of these cells. Workers in this field are likely to benefit from the application of cytochemical methods used in studies of the central nervous system and more well known endocrine/neuroendocrine cells. The localization of known peptide hormones in lung neuroendocrine cells by immunocytochemical techniques should be confirmed by various physicochemical methods and bioassay whenever possible. Identification of peptide hormones associated with lung K cells and NEBs may be helpful in elucidating the function of these cells and may provide useful markers for early detection of lung small cell carcinomas and bronchial carcinoids.

We believe on the basis of the data reviewed that a reasonable case can be made to dispel the notion that lung neuroendocrine cells are progenitors to endocrine-like lung tumors. Evidence is given to suggest that lung neuroendocrine cells are derived by differentiation from a separate population of epithelial cells which phenotypically are not neuroendocrine. It is challenging to search for the factors which control the differentiation of these cells in pulmonary epithelium. Could specific nerve endings determine that K cells form in greater numbers in the larynx and cranial aspect of the trachea than in distal airways? And why do NEBs seem to develop primarily at or near airway bifurcations? Are specific nerves associated with their development? Last, can carcinogens or other environmental agents affect neuroendocrine cells indirectly through the regulation of local or systemic factors which control neuroendocrine growth and differentiation? These are a few of the intriguing questions whose exploration we anticipate. In addition, respiratory physiologists should venture to understand the chemoreceptor function and related reflex responses associated with lung NEBs. The experimental data reviewed suggest that the NEB can respond to changes in ventilatory gas composition by altering the discharge of nerve fibers that impinge on the granulated cells of this organoid. Forebearance given to the study of lung neuroendocrine cells will eventually contribute to understanding their function in normal or diseased lungs.

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